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Drying–rewetting cycles and γ-irradiation effects on enzyme activities of distinct layers from a *Quercus ilex* L. litter

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ABSTRACT

In a Mediterranean climate, water stress is one of the principal constraints on proper forest ecosystem functioning. Drought influences rates of organic matter degradation by affecting microbial growth and enzyme activities. The objectives of this study were: (i) to evaluate the effect of repeated drying–rewetting cycles on cellulase, alkaline phosphatase and fluorescein diacetate (FDA) hydrolase activities of three distinct *Quercus ilex* L. litter layers, and (ii) to investigate the effect of these cycles on γ-irradiated litters in order to distinguish the abiotic influence on the fluctuations observed. Results, for all three layers, showed high correlations between litter water content and enzyme activities. Under mesocosm conditions, and using non-sterilized litter samples, cellulase, alkaline phosphatase, and FDA activities significantly decreased or increased during drying or rewetting cycles respectively. Significant differences were also found when evaluating the effect of litter depth on enzyme activities, the intermediate depth (OLv layer) generally being the most active. For γ-sterilized samples, FDA activity still fluctuated with drying–rewetting cycles. Assays showed that pre-humidification of γ-irradiated litter increased FDA activity two-fold in the first 30 min. All these results have shown that, following drying–rewetting cycles, some of the fluctuations occur independently of microbial growth, suggesting abiotic interactions, such as desorption, in combination with both solvatation status and conformational changes of enzymes.

1. Introduction

The global average surface temperature increased over the 20th century by about 0.6 °C and will continue to increase (IPCC, 2007). This increase in temperature may cause long periods of drought, as happened in Europe in summer 2003 (Beniston, 2003). Moreover, there are clear indications that, in the future, some regions will increasingly suffer drought effects (Calanca, 2007). In a Mediterranean climate, water stress seems to be one of the principal constraints (Sardans and Peñuelas, 2005) on the functioning of forest ecosystem biogeochemical cycles. Under these conditions, decreased moisture may considerably modify soil and litter characteristics such as microbial biomass (Bottner, 1985), organic matter quality (Denef et al., 2001), microbial functional diversity (Zak et al., 1994) and enzyme activities (Zornoza et al., 2006). On this point, Taylor (1998) clearly demonstrated that mass loss was substantially faster from fresh litter than from air-dried litter, and that applying drying–rewetting cycles (14) significantly accelerated litter decomposition.

Among the different biological parameters involved in litter mineralization, several studies have shown high correlations between enzyme activities and soil or litter moisture (Rastin et al., 1988; Yavitt et al., 2004; Sardans and Peñuelas, 2005; Niyogi and Xue, 2006). In particular, in a Mediterranean climate, moisture fluctuations considerably affect the activity and the diversity (isoenzymes) of soil and litter enzymes (Criquet et al., 2000, 2002, 2004; Fioretto et al., 2000, 2001, 2005; Sardans and Peñuelas, 2005; Zornoza et al., 2006). Moreover, a recent study (Sardans and Peñuelas, 2005) pointed out that moisture reduction has drastic effects on most enzyme activities, and that longer and more severe drought periods are therefore expected to induce slower nutrient turnover in Mediterranean forest ecosystems. However, they also mentioned that some enzyme activities, such as N mineralizing enzymes, were more severely affected by drought periods. These differences in the response of soil and litter enzyme activities to moisture fluctuations can be explained by the diversity of mineral and organic compounds which can adsorb and immobilize enzymes (Sinsabaugh et al., 1994). Such immobilization processes can affect the catalytic properties of soil enzymes (De Cesare et al., 2000),

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but also confer a longer half-life to enzymes (Burns, 1982). As a consequence of these interactions, it was reported that the affinity of enzymes changes with soil composition, including tannins, colloids and minerals (Maie et al., 2003; Marx et al., 2005). Even though enzyme activities do not respond in the same way to air-drying and rewetting cycles, the immediate effects of water on litter enzymes are still poorly described and understood. Indeed, while most of the studies mentioned showed clear correlations between enzyme activities and litter or soil water contents, they often did not reveal equally clear relationships between enzyme activities and microbial biomass following rewetting events.

Our aim here was thus double: first, to demonstrate that the relationships between enzyme activities and litter moisture observed in numerous field studies can be easily simulated in laboratory mesocosms. Second, to determine, using different time scales (d, h, min), how long it takes to observe the effects of litter rewetting on litter enzyme activities, and to what extent these effects are influenced by biotic (microbial growth and metabolism) or abiotic processes. To this end, we performed γ-irradiation in order to study the influence of the abiotic processes alone on enzyme activities, and to establish the degree of relationship between microbial growth and enzyme activities. With this second objective, we progressively focused on an activity commonly used in litter studies: FDA hydrolyzing activity. Summing up all the experiments performed during our study, we finally aimed to progressively demonstrate that some abiotic interactions can also partly explain the fluctuations in enzyme activities due to drying–rewetting events within forest litters.

2. Methods

2.1. Litter

Evergreen oak litter (*Quercus ilex* L.) was collected in March 2004 from a 1000 m^2 dense coppice at 'La Gardiole de Rians' (Var region, France), in an area with a Mediterranean-type climate. Soil of the study site is a reddish-brown fersialitic soil presenting a thick litter and a biomacro-structurated A1 horizon typical of amphimull (Tagger et al., 2008). Litter was collected randomly from a surface of 0.5 ha, and three litter layers from the O horizon (Green et al., 1993) were distinguished: the OLn layer (upper layer), formed by leaves over a period of less than 1 y, non-degraded and non-compressed; the OLv layer formed by leaves still recognizable despite decay, non-compressed; and the OF layer, formed by non-recognizable leaves and often compressed in lumps. Litter samples were characterized by Solid State ^{13}C CPMAS NMR and showed an increase in their humification index (Kögel, 1986), with values of 0.31, 0.51 and 0.56 in OLn, OLv and OF, respectively. All samples were air-dried at room temperature and stocked until utilization. 1 g of litter was weighed and dried overnight at 105 °C to calculate the dry weight. For γ-irradiation experiments, litter samples were γ-sterilized with a 33 kGy dose, and, thereafter, sterile conditions of litter materials were confirmed by plating out samples on nutrient agar. Enzyme activities were measured before and after γ-irradiation.

2.2. Enzyme assays

Unless otherwise indicated, each litter sample was ground (<0.5 mm) during 30 s (3 × 10 s) using a mixer (Moulinex, model D56, 750 W, 50–60 Hz), and enzyme activities were measured in triplicate spectrophotometrically (Kontron, model Uvikon 860). For cellulase activity measurement, 300 mg of each litter layer and 30 ml of 0.1 M acetate buffer (pH 6.0) with 1% of CarboxyMethylCellulose (CMC) solution were mixed and incubated at 50 °C for 1 h. Controls were realized without adding CMC in acetate buffer. After incubation, the sugars released from the hydrolysis of CMC were measured (Somogyi, 1952; Nelson, 1994). A calibration curve was established with glucose (0–100 μg ml^{-1}) and cellulase activity was expressed in μmole of glucose released min^{-1} (U) per g of dry weight (U g^{-1} DW).

Alkaline phosphatase activity was measured according to Tabatabai and Bremner (1969). Since our study focused mainly on microbial enzymes, this enzyme was chosen because it is absent from plant tissues (Criquet et al., 2004). The method used to measure alkaline phosphatase was based on the measurement at 412 nm of *p*-nitrophenol (*p*-NP), released during the incubation of litter with *p*-nitrophenyl phosphate mono-ester (*p*-NPP). 300 mg of ground litter and 30 ml of 0.1 M NaOH–Glycine (pH 9) buffer with 5 mM of *p*-NPP were mixed and incubated 1 h at 37 °C. A *p*-NP calibration curve (0–20 mg l^{-1}) was performed under the same conditions as the assays, and results were expressed in units defined as μmole of *p*-NP released min^{-1} (U) per g of dry weight (U g^{-1} DW). FDA activity was measured according to Schnürer and Rosswall (1982) with some modifications: 300 mg of ground litter and 30 ml of 50 mM phosphate buffer (pH 7.6) with 200 μl of FDA (Sigma Aldrich Chemical Co. Ltd.) (2 mg ml^{-1} acetone AR grade) were mixed and incubated for 30 min at 37 °C. The reaction was stopped by adding 1 ml of acetone/water solution (1:1 v/v) to 1 ml of the reaction solution and the fluorescein released was measured at 490 nm (Kontron, model Uvikon 860). Results were expressed in μmole of fluorescein released min^{-1} (U) per g of dry weight (U g^{-1} DW).

2.3. Drying–rewetting experiments

2.3.1. Experiment 1: effect of drying–rewetting cycles on enzyme activities in a mesocosm study

Experiments ($n = 4$) were performed in 30 L mesocosms (polyethylene rectangular tanks, 40 cm long × 30 cm wide × 25 cm deep). Three litter layers (100 g of each) were placed into mesocosms which were previously filled with 2 kg of a rendzine soil, collected under the litter layers of the same sampling plot. Each layer was separated by a plastic grid (squares of 0.5 cm^2) to avoid mixing levels during sampling. Each layer was placed according to its forest floor location: OF (bottom layer), OLv (middle layer) and OLn (top layer), respectively. All mesocosms were incubated inside laboratory and covered with a transparent, perforated polyethylene film, to allow air-drying and gas exchanges during the experiment. Drying–rewetting experiments were performed over 32 d for OLn and OLv layers, and over 100 d for OF layer because of its longer natural drying time. For all litter layers considered, three drying–rewetting cycles were completed during the experiment, and samples of the different layers were regularly collected (4 for each layer and each sampling date) and analyzed for enzyme activities described previously.

2.3.2. Experiment 2: short-term effects of drying–rewetting cycles on enzyme activities of γ-irradiated litters

Litter samples from the different layers were irradiated as described above, in order to monitor fluctuations in enzyme activities subsequent to drying–rewetting, without microorganisms proliferating in the system. As this experiment was intended to determine whether fluctuations in enzyme activities also occur during short incubations, FDA activity was taken as a model, and drying–rewetting cycles were accelerated by separately placing leaves from the different layers in a funnel, which allowed very quick drying of leaf materials compared to mesocosms.

2.3.3. Experiment 3: effect of pre-moistening of γ-irradiated litter on kinetics of FDA hydrolysis

On the basis of the same dry weight, dried and pre-moistened litter samples (300 mg DW each) were used to measure FDA activity according to the protocol described previously. Leaves of the OLv layer were taken as a model and kinetics of FDA hydrolysis in both

dried and pre-moistened samples were directly monitored over 150 min.

2.4. Statistical analyses

Means and standard error of means were determined for each set of replicates. Analyses of variance (ANOVA) were carried out, and the means were compared using least significant differences (LSD) test, with a significance level of $P < 0.05$. All calculations were performed using Statistica software version 6.0 (StatSoft, Maisons-Alfort, France).

3. Results

3.1. Experiment 1: effect of drying–rewetting cycles on enzyme activities in mesocosms

During the 1st experiment, moisture measurements from the different layers revealed increased water content with depth, thus indicating increased water holding capacities (WHC) during the course of litter degradation. For example, Fig. 1 shows that water contents after the 1st rewetting were 51.7%, 68.2% and 69.3% in the OLn, OLv and OF layers respectively. After the 1st rewetting cycle, the OLn layer showed the fastest loss of water (slope = −4.46), followed by the OLv (slope = −3.45) and the OF respectively (slope = −0.09). Humidity in the OF layer remained unchanged over 27 d, showing the greater capacity of this layer to retain water. It therefore appeared necessary to increase the incubation time to 100 d in order to complete 3 drying–rewetting cycles in this layer. The last two drying phases appeared to be more rapid, probably because the air-temperature increased daily (data not shown) during the experiment. Results of the experiment on repeated drying–rewetting cycles (3) revealed significant effects of moisture, as well as litter depth (layers), on all the enzymes considered (Table 1). Hence, drying–rewetting cycles significantly affected the different activities, and detailed results for each enzyme are given below. Almost all the enzymes measured in all layers tended to decrease to a greater or a lesser extent following the successive drying–rewetting cycles.

In each litter layer, cellulase activity fluctuated significantly with moisture, showing correlation coefficients of 0.68, 0.87 and 0.78 ($P < 0.05$) in the OLn, the OLv and the OF layers respectively (Table 2). Rewetting events always induced peaks in cellulase activities, which occurred in the sample immediately (1 d) after water addition. The response of cellulase activity (Fig. 1) to rewetting varied in its intensity according to layer and rewetting date.

The greatest increase (+810%) was observed in the OLv layer, which was also the layer with the highest cellulase activities. On the other hand, the lowest activities were detected in the deepest layer (OF), yet they also fluctuated greatly in drying–rewetting, ranging from +57% to +536%. During the first 30 d, the moisture of the deepest (OF) layer changed only slightly, due to its capacity to retain water, and cellulase activity was generally stable around a mean value of 0.3 U g^{-1} DW.

Phosphatase activities (Fig. 2) increased progressively with litter depth, ranging from 0.15 to 0.41 U g^{-1} DW in the upper (OLn) layer, from 0.21 to 0.51 U g^{-1} DW in the OLv layer, and from 0.19 to 0.59 U g^{-1} DW in the deepest (OF) layer. Following the successive drying–rewetting cycles, alkaline phosphatase activities showed similar patterns to cellulases in their dynamics. Therefore, phosphatase activities were significantly correlated with moisture, since they showed correlation coefficients of 0.71, 0.76 and 0.82 ($P < 0.05$) in the OLn, the OLv and the OF layers respectively. However, the responses of phosphatase activities were much less intense than those of cellulases after rewetting. Indeed, increases in their activities ranged between +20% and +96%, with the OLv layer showing the greatest increases and the OF showing the smallest. It can also be observed that both the baseline of phosphatase activities, corresponding to dried samples, and the peaks of activities tended to decrease during the experiment. This was also observed for the other enzymes and may be explained by losses in litter enzymes due to leaching that may have occurred during rewetting of the different

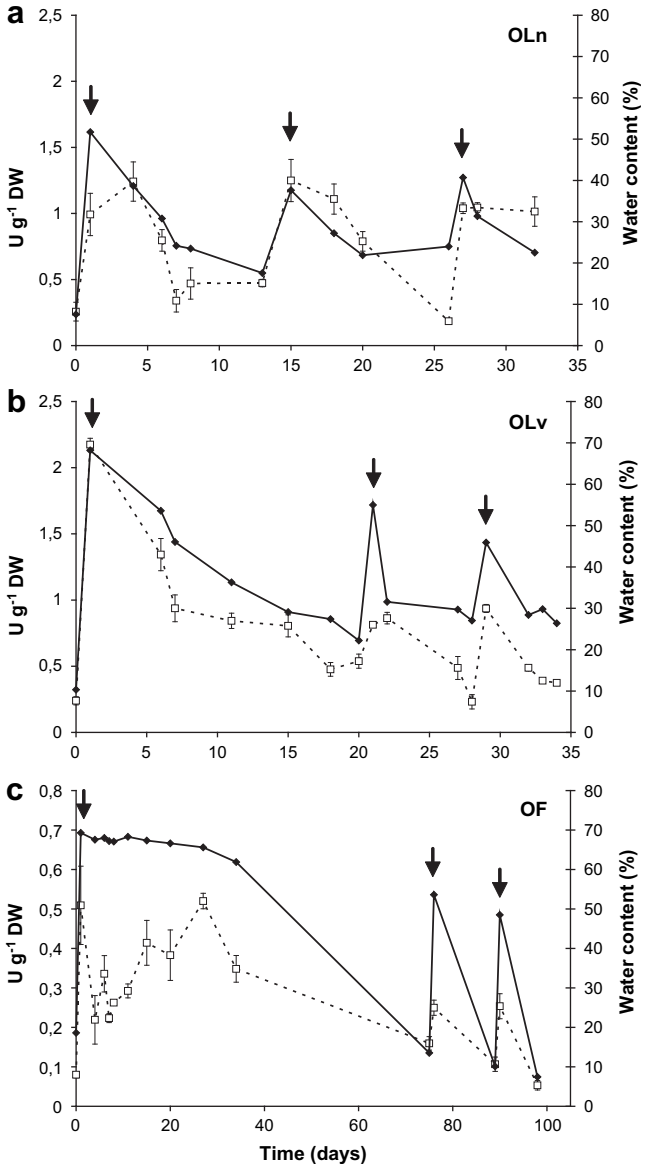

Fig. 1. Response of cellulase activity to drying–rewetting cycles, in the three litter layers evaluated: OLn, OLv and OF. Mean values of three replicates ($n = 3$). Bars are standard error. Arrows indicate rewetting events. —□—: cellulase activity. —◆—: water content.

Table 1
Two-way ANOVA results on the effects of depth, water treatment and depth × water treatment on FDA, cellulase (Cell) and alkaline phosphatase (AlP) activities.

Effect	df	*F* values		
		Cell	AlP	FDA
Depth	2	216***	27***	49***
Water	1	40***	53***	100.3***
Depth × water	2	0^{NS}	6.6**	7.6***

***: Significant at $P < 0.001$.
**: Significant at $P < 0.05$. NS: Non-significant.

Table 2
Correlation coefficients between enzyme activities and moisture (all $P < 0.05$). Results from field and mesocosm experiments in the different litter layers: whole, OLn, OLv and OF.

Enzyme	Litter	*r*	Conditions	References
Cellulase	Whole	0.73	Field	Fioretto et al. (2001)
	OLn	0.68	Mesocosm	This work
	OLv	0.87	Mesocosm	This work
	OF	0.78	Mesocosm	This work
	Whole	0.86	Field	Nèble (2005)
	OLv	0.84	Field	Criquet et al. (2002)
Acid phosphatase	OLv	0.72	Field	Criquet et al. (2004)
Alkaline phosphatase	Whole	0.55	Mesocosm	Nèble et al. (2007)
	OLn	0.71	Mesocosm	This work
	OLv	0.76	Mesocosm	This work
	OF	0.82	Mesocosm	This work
FDA hydrolase	OLn	0.67	Mesocosm	This work
	OLv	0.78	Mesocosm	This work
	OF	0.8	Mesocosm	This work

layers. The last enzyme activity measured during this study, FDA hydrolase (Fig. 3), also fluctuated significantly with moisture, showing correlation coefficients of 0.67, 0.78 and 0.80 ($P < 0.05$) in the OLn, the OLv and the OF layers respectively. However, the dynamic patterns of this activity differed slightly from the two others after rewetting. Indeed, although the rapid effect of rewetting on FDA activities was obvious after the last two cycles in the different layers, no rapid effect was observed following the first cycle. Increases in FDA activities after the last two rewetting cycles ranged between +3% and +286%, the OLv layer being more sensitive to litter moisture fluctuations than the OF layer.

Results from these studies indicate that the correlations obtained during the present experiment are consistent with the correlations observed under field conditions, and that mesocosms can thus be used successfully to simulate drying–rewetting cycles under laboratory conditions (Table 2).

3.2. Experiment 2: short-term effects of drying–rewetting cycles on enzyme activities of γ-irradiated litters

The OLv layer generally exhibited the highest level of activities, as previously described in the first experiment. As a consequence, we chose the OLv layer for the preliminary study on the denaturing effects of γ-irradiation on the three enzymes. Statistical analyses showed that the FDA was the sole activity totally unaffected by γ-irradiation, whereas cellulase and alkaline phosphatase activities decreased by 39% and 20% respectively after γ-irradiation (Fig. 4). When the effect of γ-irradiation on the FDA activity was compared in the three litter layers (Fig. 5), results showed that the activity was unaffected by the treatment ($P < 0.01$). As a consequence, and in order to simplify the experimental design, FDA activity was chosen as a model for the rest of the experiments involving γ-irradiation.

Fig. 6 shows FDA activities monitored during two drying–rewetting cycles in the litter layers that were γ-irradiated. Overall, FDA activities from the γ-irradiated litters responded with patterns similar to those of non-irradiated litter layers previously described. Indeed, FDA activities still fluctuated according to moisture in γ-irradiated litters, showing correlation coefficients of 0.43, 0.74 and 0.98 in the OLn, the OLv and the OF layers respectively. With the exception of the OLn layer, correlation coefficients indicated that drying–rewetting effects on FDA activities were largely maintained even when microbial growth was inhibited. Variations in intensity of FDA activities following rewetting events ranged between +11.34 mU and +11.73 mU in the OLn layer, between +11.45 mU and +19.12 mU in the OLv layer and between +45.2 mU and +50.6 mU in the OF layer. For the non-γ-sterilized litter (Fig. 3), these variations ranged between +4.65 mU and +33.8 mU in the OLn layer, between +59.2 mU and +59.6 mU in the OLv layer (the value from the 1st cycle was not considered because of a probable leaching phenomenon), and between +1.32 mU and +25.68 mU in the OF layer. Thus, and as will be discussed hereunder, it appeared that γ-irradiation showed different effects on the dynamics of FDA activities depending on the litter layer considered.

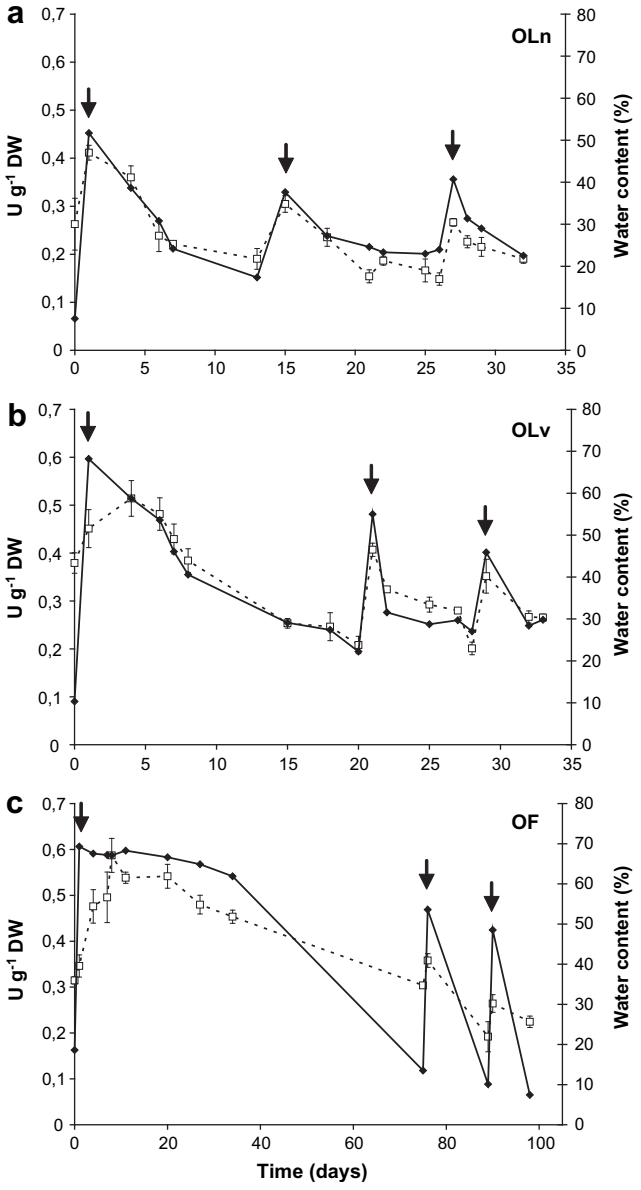

Fig. 2. Response of alkaline phosphatase activity to drying–rewetting cycles, in the three litter layers evaluated: OLn, OLv and OF. Mean values of three replicates ($n = 3$). Bars are standard error. Arrows indicate rewetting events. —□—: alkaline phosphatase activity. ——◆——: water content.

3.3. Experiment 3: effect of pre-moistening of γ-irradiated litter on kinetics of FDA hydrolysis

Fig. 7 shows the kinetic of fluorescein released from dried and pre-moistened OLv γ-irradiated litter incubated with buffered FDA. It can be observed that the rate of FDA hydrolysis by litter enzymes was faster in pre-moistened samples than in dried samples. Indeed,

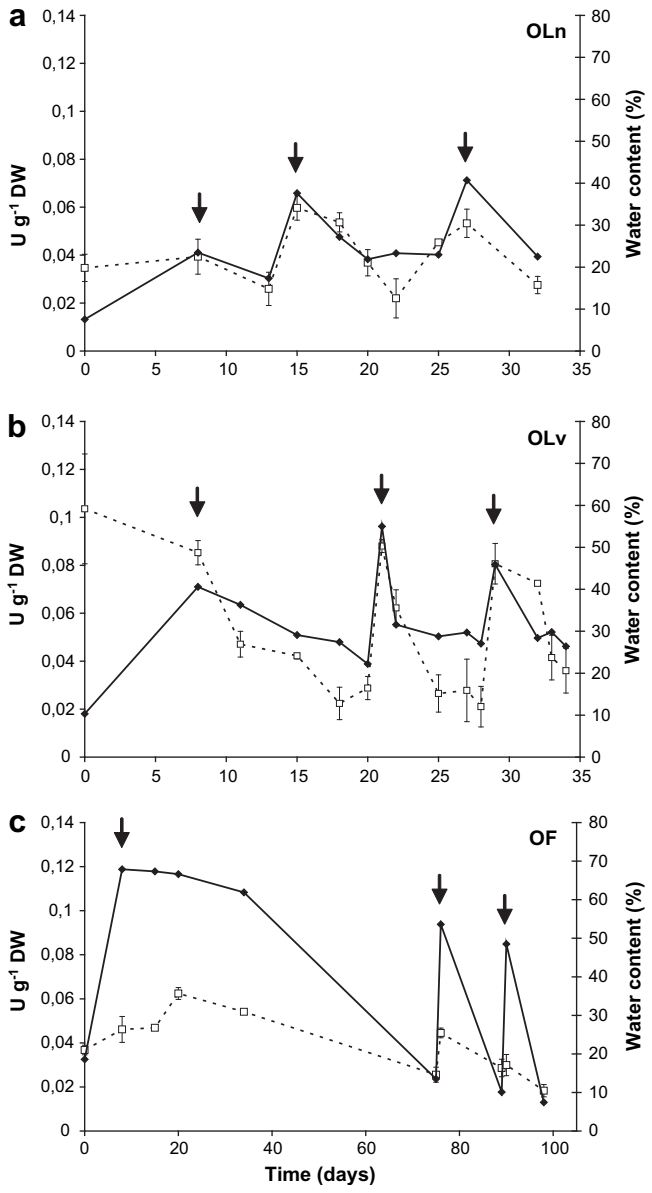

Fig. 3. Response of FDA hydrolase activity to drying–rewetting cycles, in the three litter layers evaluated: OLn, OLv and OF. Mean values of three replicates ($n = 3$). Bars are standard error. Arrows indicate rewetting events. —□—: FDA activity. ——◆——: water content.

after 30 min reaction, fluorescein released in pre-moistened samples was twice as high as in dried samples. After 1 h of reaction, both dried and pre-moistened samples reached the same stationary phase in fluorescein release.

4. Discussion

In earlier studies, we demonstrated the influence of abiotic factors such as temperature (Alarcon-Gutierrez et al., 2008a), N availability (Alarcón-Gutiérrez et al., 2008b) and/or P availability (unpublished data) on the microbial functions varied considerably with the stage of degradation and therefore organic matter (OM) litter quality. Here, therefore, we used three litter layers of differing OM quality, *i.e.* with increased humification index, depending on their litter depth, to study the effect of dry–wet cycles on litter enzyme activities.

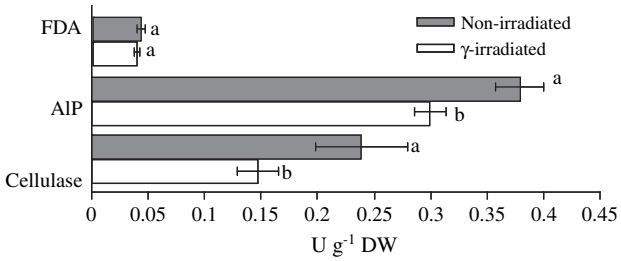

Fig. 4. Response of FDA hydrolase, alkaline phosphatase and cellulase activities to γ-irradiation of the OLv litter layer. Mean values of five replicates ($n = 5$). Bars are standard error. Means with the same letter are not significantly different using a one-way ANOVA with LSD test. $P < 0.01$.

Our results show that the different layers of the evergreen oak litter exhibit increased water holding capacities (WHC) with depth (see *e.g.* Fig. 1). Moreover, their drying rates are also different, the more humified litter layer (OF) showing the greatest capacity to retain water. These observations are consistent with the work of Taylor and Parkinson (1988), where the same characteristics were observed in the case of lodgepole-jack pine and trembling aspen litters. They can be explained by variations in OM characteristics of litter layers, such as contents in hydrophobic (*e.g.* wax and cutin) or hydrophilic (*e.g.* amorphous cellulose) compounds, which are known to influence water absorption capacities (Kunst and Samuels, 2003; Alarcon-Gutierrez et al., 2009). Our preliminary results have indicated that litter OM quality had significant effects on both WHC and enzyme activities. Thus, before investigating the abiotic effect of dry–wet cycles on enzyme activities we decided to check whether the enzyme activity fluctuations observed under field conditions (Criquet et al., 2000, 2002, 2004; Fioretto et al., 2000, 2001, 2005; Sardans and Peñuelas, 2005; Zornoza et al., 2006) were reproducible under laboratory conditions. Results (Figs. 1–3) show that, in our laboratory mesocosms, cellulase, alkaline phosphatase (AlP) and FDA activities of non-sterilized litter layers were significantly affected by drying–rewetting cycles, and responses to rewetting were generally rapid (1 d). Similar data have been obtained in other studies investigating the relationships between moisture and cellulase and phosphatase activities of various Mediterranean forest litters (Fioretto et al., 2001; Criquet et al., 2002, 2004; Nèble, 2005; Sardans and Peñuelas, 2005; Doyle et al., 2006; Nèble et al., 2007). Moreover, fluctuations in enzyme activities due to moisture conditions are not restricted to litter systems, since these kinds of relationships have also been observed in soils (Clein and Schimel, 1994; Hinojosa et al., 2004). Thus, our results indicate that the correlation obtained is consistent with correlations found in other field studies, and that the use of mesocosms can efficiently reproduce the effects

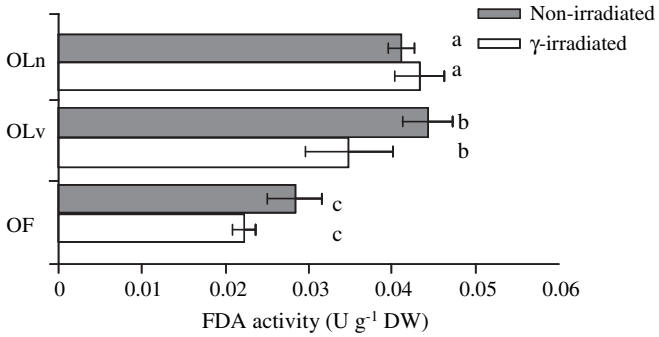

Fig. 5. Effect of γ-irradiation on the FDA activity of the three litter layers (OLn, OLv and OF). Mean values of five replicates ($n = 5$). Bars are standard error. Means with the same letter are not significantly different using a one-way ANOVA with LSD test. $P < 0.01$.

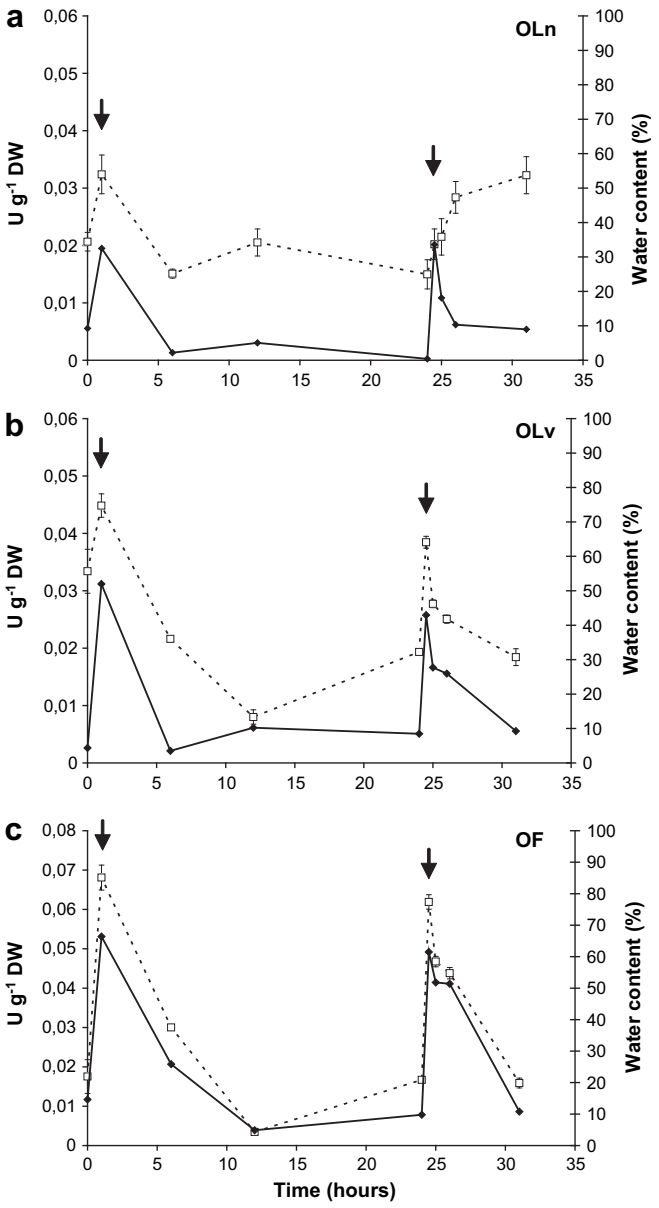

Fig. 6. Effect of the drying–rewetting cycles on the FDA activity of the γ-irradiated OLv layer. Mean values of three replicates ($n = 3$). Bars are standard error. Arrows indicate rewetting events. —□—: FDA activity. ——◆——: water content.

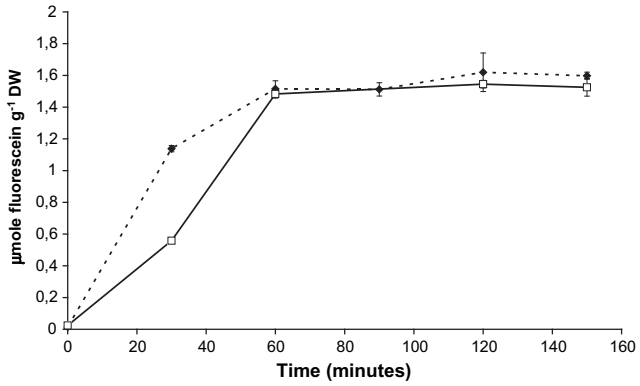

Fig. 7. Effect of litter pre-moistening on the FDA activity of the γ-irradiated OLv layer. Mean values of five replicates ($n = 5$). Bars are standard error. —□—: humid. ——◆——: dried.

of natural drying–rewetting cycles on litter enzyme activities (Table 2). In the literature, all these enzyme variations were observed in long-term field experiments, and it was argued that variations were due to microbial activity (Fioretto et al., 2001, 2005; Criquet et al., 2002; Doyle et al., 2006) or to differences in enzyme sensitivity to drying depending on their microbial origin (*i.e.* fungi or bacteria) (Niemi et al., 2007).

However, these studies found no or low correlations between microbial biomasses and enzyme activities (Fioretto et al., 2001; Criquet et al., 2002, 2004) for *Cistus incanus* and *Q. ilex* litters respectively. This absence of relationships between microbial biomass (*i.e.* bacteria and fungi) and enzyme activities during drying–rewetting cycles was also observed in our mesocosm study (data not shown). Hence, in order to determine whether enzyme activity can fluctuate independently of microbial growth, litter samples were γ-irradiated and thereafter subjected to drying–rewetting cycles. During the course of this 2nd experiment, FDA hydrolases appeared the most stable enzymes under γ-irradiation since their activity was not significantly affected by this sterilization treatment (Figs. 4 and 5). Conversely, phosphatase and cellulase activities were significantly decreased by γ-irradiation, probably due to enzyme structure disorganization, as suggested by Constantinovici et al. (2009). Other previous studies have also reported quite different effects (*i.e.* no, stimulating or inhibitory) of γ-rays on several different enzymes (Vasileva-Tonkova and Chomoneva, 2004; Gralik and Warchalewski, 2006; Constantinovici et al., 2009). Thus, the apparent lack of effect of γ-irradiation on litter FDA hydrolyzing activities could be explained by a composite response from a large pool of different enzymes with different sensitivities towards γ-rays, which smoothes out responses, leading to an apparent zero effect. Indeed, FDA is known to be a non-specific enzymatic substrate that can be hydrolyzed by a number of different enzymes such as esterases, proteases, lipases and cutinases (Alarcón-Gutiérrez et al., 2008b), and which can react quite differently when γ-irradiated. Such differences in enzyme resistance to irradiation were also observed by Lensi et al. (1991), who argued that the location of enzymes in soil could also be considered as a factor controlling resistance to γ-rays and to their subsequent activity. The resistance to γ-irradiation of FDA hydrolyzing enzymes led us to select this activity as a model for the rest of our study involving γ-sterilized litters. Results show that, over a short-term experiment (30 h) comprising two drying–rewetting cycles, enzyme activities of γ-sterilized litters still fluctuated widely depending on the water content of the litter (Fig. 6). This was especially true for the two deepest layers (OLv and OF), although it was less clear for the upper layer (OLn). As mentioned in a previous study (Alarcón-Gutiérrez et al., 2008b), the occurrence of substances interfering with FDA assay in the OLn layer may explain the lower correlation observed. Considering the intensity of the responses of FDA hydrolases following rewetting cycles, it appeared that γ-irradiation had different effects as a function of litter organic matter quality. Indeed, in some cases, enzyme activity increases were greater in non-sterilized than in γ-ray sterilized samples, suggesting a larger contribution of biotic factors (enzyme synthesis) to the fluctuations observed. This was particularly obvious in the case of the OLv layer, where the fluctuations in FDA activities were about 3.9 lower in the irradiated litter than in the native litter. This result appears coherent since, during this study as well as in some of our previous work, we clearly demonstrated that this layer was biologically the most active (Alarcon-Gutierrez et al., 2008a,c, 2009). However, in the more humified layer (OF), patterns in intensity responses were different, since fluctuations in FDA activities were greater in γ-sterilized samples than in untreated ones. In this case, the result may reflect a larger

contribution of abiotic factors to the dynamics observed, as a function of litter OM quality. This difference may be explained by modifications in litter constituents irradiated with γ-rays. Indeed, γ-rays are known to potentially induce several effects on organic molecules, such as changes in protein conformation (Vasileva-Tonkova and Chomoneva, 2004), hydrolysis of chemical bonds, thereby cleaving molecules (Gralik and Warchalewski, 2006) and modifying OM quality, and decreasing adsorption properties of organic polymers including those naturally found in litters (Vazquez et al., 2005). Thus, it can be hypothesized that the abiotically exacerbated FDA activities observed in the irradiated OF layer partly originate from the effects of γ-rays on litter OM in desorbing and/or enhancing activities of FDA hydrolases. Finally, in a 3rd and final experiment, also performed on γ-sterilized litter, duration was further reduced (min) and FDA hydrolyzing kinetics of both dried and rewet samples were monitored. The results of this final experiment indicate that hydrolyzing rate was considerably higher in rewet samples than in dried samples (Fig. 7).

Results from all these experiments suggest that some of the enzyme fluctuations observed in litter may originate from abiotic processes. Increased enzyme activities may originate from dead cells, due to microbial damage caused by drying-rewetting cycles (Salonius, 1983; Van Gestel et al., 1993; Hinojosa et al., 2004). However, the qualitative fluctuations observed during this work in both non-irradiated and γ-irradiated samples, where dead cells did not usually affect enzyme activities, were similar. This suggests that other factors like desorption of enzymes, hydrophilic enzyme affinity, rehydration and enzyme conformational changes (Noinville et al., 2004), and the formation of complexes between proteins and organic components (*e.g.* tannins, humic acids) could be responsible for fluctuations in enzyme activities of litter. In this respect, some studies have shown that the enzyme activity (*i.e.* acid phosphatase) changes according to the enzyme binding affinity to various colloidal particles of soil (Huang et al., 2005). These studies have shown that fine organic particles with a large cation exchange capacity adsorb more proteins, thus reducing their activities. Another explanation could be that a non-aqueous environment has a variety of effects on enzymes: dissociation of sub-units, shift in enzyme conformation equilibrium, dehydration of enzymes and decrease in intra-molecular motions, affecting the rate of catalytic reactions in several different ways (Bell et al., 1997; Lind et al., 2004; Zappa et al., 2004). Moreover, microorganisms can adapt their protein when subjected to extreme environmental conditions (Brown, 1976) such as water stress and low a_w. In a Mediterranean climate, it can be hypothesized that enzymes are suited to this water stress, reacting fast to a change in moisture conditions. However, all these hypotheses require further investigations, and would appear to us an obvious avenue for future research.

5. Conclusion

The fluctuations in litter enzyme activities observed in a Mediterranean climate in relation to litter water content may not be exclusively due to the stimulation of soil biotic processes. This study has shown that, following drying–rewetting cycles, some of the fluctuations occur independently of microbial growth, suggesting abiotic interactions. It may be that desorption or hydration result in conformational changes in enzymes; but of course this alone does not explain all the variations in the enzyme activities observed throughout a one-year field experiment. Moreover, we assume that litter enzymes are continually influenced by adsorption and by the continuous neosynthesis and proteolysis from microorganisms. These interactions need to be quantified more precisely, and we suggest that future work could focus on such aspect of litter enzymology by investigating a larger set of enzyme activities. What this study has demonstrated is that moisture probably represents a "background noise" during enzyme activity measurement, and that it may be more appropriate to work with dried samples or at their WHC, in order to distinguish the influence of other factors on litter functioning.

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References

Alarcon-Gutierrez, E., Couchaud, B., Augur, Christopher., Calvert, V., Criquet, S., 2008a. Effect of nitrogen availability on microbial activities, densities and functional diversities involved in the degradation of a Mediterranean evergreen oak litter (*Quercus ilex* L.). Soil Biology & Biochemistry 40, 1654–1661.

Alarcón-Gutiérrez, E., Floch, C., Ruaudel, F., Criquet, S., 2008b. Non-enzymatic hydrolysis of fluorescein diacetate (FDA) in a Mediterranean oak (*Quercus ilex* L.) litter. European Journal of Soil Science 59, 139–146.

Alarcon-Gutierrez, E., Floch, C., Ziarelli, F., Albrecht, R., Le Petit, J., Augur, C., Criquet, S., 2008c. Characterization of a Mediterranean litter by 13C CPMAS NMR: relationships between litter depth, enzyme activities and temperature. European Journal of Soil Science 59, 486–495.

Alarcon-Gutierrez, E., Floch, C., Augur, C., Le Petit, J., Ziarelli, F., Criquet, S., 2009. Spatial variations of chemical composition, microbial functional diversity, and enzyme activities in a Mediterranean litter (*Quercus ilex L.*) profile. Pedobiologia 52, 387–399.

Bell, G., Janssen, A.E.M., Halling, P.J., 1997. Water activity fails to predict critical hydration level for enzyme activity in polar organic solvents: interconversion of water concentrations and activities. Enzyme & Microbial Technology 20, 471–477.

Beniston, M., 2003. The heat wave in Europe: a shape of things to come? An analysis based on Swiss climatological data and model simulations. L02202. Geophysical Research Letters 31 doi: 02210.01029/02003GL018857.

Bottner, P., 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with 14C- and 15N-labelled plant material. Soil Biology & Biochemistry 17, 329–337.

Brown, A.D., 1976. Microbial water stress. Bacteriology Review 40, 803–846.

Burns, R.G., 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biology & Biochemistry 14, 423–427.

Calanca, P., 2007. Climate change and drought occurrence in the Alpine region: how severe are becoming the extremes? Global & Planetary Change 57, 151–160.

Clein, J.S., Schimel, J.P., 1994. Reduction in microbial activity in birch litter due to drying and rewetting event. Soil Biology & Biochemistry 26, 403–406.

Constantinovici, M., Oancea, D., Zaharescu, T., 2009. Gamma irradiation effect on the enzymatic activities of horseradish and apple peroxidases. Radiation Physics & Chemistry 78, 33–36.

Criquet, S., Farnet, A.M., Tagger, S., Le Petit, J., 2000. Annual variations of phenoloxidase activities in an evergreen oak litter: influence of certain biotic and abiotic factors. Soil Biology & Biochemistry 32, 1505–1513.

Criquet, S., Tagger, S., Vogt, G., Le Petit, J., 2002. Endoglucanase and β-glycosidase activities in an evergreen oak litter: annual variation and regulating factors. Soil Biology & Biochemistry 34, 1111–1120.

Criquet, S., Ferre, E., Farnet, A.M., Le Petit, J., 2004. Annual dynamics of phosphatase activities in an evergreen oak litter: influence of biotic and abiotic factors. Soil Biology & Biochemistry 36, 1111–1118.

De Cesare, F., Garzillo, A.M., Buonocore, V., Badalucco, L., 2000. Use of sonication for measuring acid phosphatase activity in soil. Soil Biology & Biochemistry 32, 825–832.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology & Biochemistry 33, 1599–1611.

Doyle, J., Pavel, R., Barness, G., Steinberger, Y., 2006. Cellulase dynamics in a desert soil. Soil Biology & Biochemistry 38, 371–376.

Fioretto, A., Papa, S., Curcio, E., Sorrentino, G., Fuggi, A., 2000. Enzyme dynamics on decomposing leaf litter of *Cistus incanus* and *Myrtus communis* in a Mediterranean ecosystem. Soil Biology & Biochemistry 32, 1847–1855.

Fioretto, A., Papa, S., Sorrentino, G., Fuggi, A., 2001. Decomposition of *Cistus incanus* leaf litter in a Mediterranean maquis ecosystem: mass loss, microbial enzyme activities and nutrient changes. Soil Biology & Biochemistry 33, 311–321.

Fioretto, A., Di Nardo, C., Papa, S., Fuggi, A., 2005. Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. Soil Biology & Biochemistry 37, 1083–1091.

Gralik, J., Warchalewski, J.R., 2006. The influence of g-irradiation on some biological activities and electrophoresis patterns of wheat grain albumin fraction. Food Chemistry 99, 289–298.

Green, R.N., Trowbridge, R.L., Klinka, K., 1993. Toward a taxonomic classification of humus forms. Forest Science, Monograph 29, 1–49.

Hinojosa, M.B., Carreira, J.A., Garcia-Ruiz, R., Dick, R.P., 2004. Soil moisture pre-treatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils. Soil Biology & Biochemistry 36, 1559–1568.

Huang, Q., Liang, W., Cai, P., 2005. Adsorption, desorption and activities of acid phosphatase on various colloidal particles from an Ultisol. Colloids & Surface B – Biointerfaces 45, 209–214.

IPCC Climate Change, 2007. Impacts, adaptations and vulnerability. In: Parry, M.L., Canziani, O.F., Palutikof, J.P., Van Der Linden, P.J., Hanson, C.E. (Eds.), Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, p. 976.

Kunst, L., Samuels, A.L., 2003. Biosynthesis and secretion of plant cuticular wax. Progress in Lipid Research 42, 51–80.

Kögel, I., 1986. Estimation and decomposition pattern of the lignin component in forest humus layers. Soil Biology & Biochemistry 18, 589–594.

Lensi, R., Lescure, C., Steinberg, C., Savoie, J.M., Faurie, G., 1991. Dynamics of residual enzyme activities, denitrification potential, and physico-chemical properties in a γ-sterilized soil. Soil Biology & Biochemistry 23, 367–373.

Lind, P.A., Daniel, R.M., Monk, C., Dunn, R.V., 2004. Esterase catalysis of substrate vapour: enzyme activity occurs at very low hydration. Biochimica & Biophysica Acta 1702, 103–110.

Maie, N., Behrens, A., Knicker, H., Kogel-Knabner, I., 2003. Changes in the structure and protein binding ability of condensed tannins during decomposition of fresh needles and leaves. Soil Biology & Biochemistry 35, 577–589.

Marx, M.C., Kandeler, E., Wood, M., Wermbter, N., Jarvis, S.C., 2005. Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biology & Biochemistry 37, 35–48.

Nèble, S., 2005. Effets de l'épandage de boues de station d'épuration sur l'évolution des caractéristiques chimiques, microbiologiques et enzymatiques d'une litière de chêne-liège (*Quercus suber* L.) en milieu sylvo-pastoral. Ph.D. thesis, Paul Cézanne University, Marseille, France.

Nelson, M., 1994. Photometric adaptation of Somogyi method for determination of glucose. Journal of Biology & Chemistry 153, 375–380.

Niemi, R.M., Vepsäläinen, M., Erkomaa, K., Ilvesniemi, H., 2007. Microbial activity during summer in humus layers under *Pinus silvestris* and *Alnus incana*. Forest Ecology & Management 242, 314–323.

Niyogi, D., Xue, Y., 2006. Soil moisture regulates the biological response of elevated atmospheric CO_2 concentrations in a coupled atmosphere biosphere model. Global and Planetary Change 54, 94–108.

Noinville, S., Revault, M., Quiquampoix, H., Baron, M.H., 2004. Structural effects of drying and rehydration for enzymes in soils: a kinetics-FTIR analysis of a-chymotrypsin adsorbed on montmorillonite. Journal of Colloid & Interface Science 273, 414–425.

Nèble, S., Calvert, V., Le Petit, J., Criquet, S., 2007. Dynamics of phosphatase activities in a cork oak litter (*Quercus suber* L.) following sewage sludge application. Soil Biology & Biochemistry 39, 2735–2742.

Rastin, N., Rosenplanter, K., Huttermann, A., 1988. Seasonal variation of enzyme activity and their dependence on certain soil factors in a beech forest soil. Soil Biology & Biochemistry 20, 637–642.

Salonius, P.O., 1983. Effects of air drying on the respiration of forest soil microbial populations. Soil Biology & Biochemistry 15, 199–203.

Sardans, J., Peñuelas, J., 2005. Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. forest. Soil Biology & Biochemistry 37, 455–461.

Schnürer, J., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Applied & Environmental Microbiology 43, 1256–1261.

Sinsabaugh, R.L., Moorhead, D.L., Linkins, A.E., 1994. The enzymic basis of plant litter decomposition: emergence of an ecological process. Applied Soil Ecology 1, 97–111.

Somogyi, M., 1952. Notes on sugar determination. Journal of Biology & Chemistry 95, 19–23.

Tabatabai, M.A., Bremner, J.M., 1969. Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biology & Biochemistry 1, 301–307.

Tagger, S., Périssol, C., Criquet, S., Aubert, G., Neville, P., Le Petit, J., Toutain, F., 2008. Characterization of an amphimull under Mediterranean evergreen oak forest (*Quercus ilex*): micromorphological and biodynamic descriptions. Canadian Journal of Forest Research 38, 268–277.

Taylor, B.R., 1998. Air-drying depresses rates of leaf litter decomposition. Soil Biology & Biochemistry 30, 403–412.

Taylor, B.R., Parkinson, D., 1988. Does repeated wetting and drying accelerate decay of leaf litter? Soil Biology & Biochemistry 20, 647–656.

Van Gestel, M., Merckx, R., Vlassak, K., 1993. Microbial biomass and activity in soils with fluctuating water contents. Geoderma 56, 617–626.

Vasileva-Tonkova, E., Chomoneva, T., 2004. Effect of gamma-irradiation on guanyl specific ribonuclease frm Trichoderma harzianum. Process Biochemistry 39, 2257–2260.

Vazquez, M.I., de Lara, R., Galan, P., Benavente, J., 2005. Modification of cellulosic membranes by γ-irradiation: effect on electrochemical parameters and protein adsorption. Colloids and Surfaces A: Physicochemical & Engineering Aspects 270–271, 245–251.

Yavitt, J.B., Wright, S.J., Wieder, R.K., 2004. Seasonal drought and dry-season irrigation influence leaf-litter nutrients and soil enzymes in a moist, lowland forest in Panama. Austral Ecology 29, 177–188.

Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G., 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biology & Biochemistry 26, 1101–1108.

Zappa, S., Boudrant, J., Kantrowitz, E.R., 2004. *Pyrococcus abyssi* alkaline phosphatase: the dimer is the active form. Journal of Inorganic Biochemistry 98, 575–581.

Zornoza, R., Guerrero, C., Mataix-Solera, J., Arcenegui, V., Garcia-Orenes, F., Mataix-Beneyto, J., 2006. Assessing air-drying and rewetting pre-treatment effect on some soil enzyme activities under Mediterranean conditions. Soil Biology & Biochemistry 38, 2125–2134.